Rapid fibroblast removal from high density human embryonic stem cell cultures.

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Public Summary:
Although several techniques are available for purifying the human embryonic stem cell (hESC) colonies from mouse feeder cells, these techniques are labor intensive, costly and/or destructive to the hESC. The aim of this project was to invent a method of purification that enables the harvesting of a purer population of hESCs. We have observed that in a confluent hESC culture, the MEF population can be removed using a simple and rapid aspiration of the MEF sheet.

Scientific Abstract:
Mouse embryonic fibroblasts (MEFs) were used to establish human embryonic stem cells (hESCs) cultures after blastocyst isolation(1). This feeder system maintains hESCs from undergoing spontaneous differentiation during cell expansion. However, this co-culture method is labor intensive, requires highly trained personnel, and yields low hESC purity(4). Many laboratories have attempted to minimize the number of feeder cells in hESC cultures (i.e. incorporating matrix-coated dishes or other feeder cell types(5-8)). These modified culture systems have shown some promise, but have not supplanted the standard method for culturing hESCs with mitomycin C-treated mouse embryonic fibroblasts in order to retard unwanted spontaneous differentiation of the hESC cultures. Therefore, the feeder cells used in hESC expansion should be removed during differentiation experiments. Although several techniques are available for purifying the hESC colonies (FACS, MACS, or use of drug resistant vectors) from feeders, these techniques are labor intensive, costly and/or destructive to the hESC. The aim of this project was to invent a method of purification that enables the harvesting of a purer population of hESCs. We have observed that in a confluent hESC culture, the MEF population can be removed using a simple and rapid aspiration of the MEF sheet. This removal is dependent on several factors, including lateral cell-to-cell binding of MEFs that have a lower binding affinity to the styrene culture dish, and the ability of the stem cell colonies to push the fibroblasts outward during the generation of their own "niche". The hESC were then examined for SSEA-4, Oct3/4 and Tra 1-81 expression up to 10 days after MEF removal to ensure maintenance of pluripotency. Moreover, hESC colonies were able to continue growing from into larger formations after MEF removal, providing an additional level of hESC expansion.